# Imitation of the Ion Diffusion and Ion Pumping Mechanisms of a Living Cell by Nanoporous Alumina Membrane

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# IMITATION OF THE ION DIFFUSION AND ION PUMPING MECHANISMS OF A LIVING CELL BY NANOPOROUS ALUMINA MEMBRANE

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## ABSTRACT

Diffusion and pumping of ions are two major mechanisms of the ion channels in real cell membranes. The main goal of this research is to imitate the ion diffusion and pumping properties of a real cell membrane using the inorganic porous alumina membrane. The diameters of the pores on the membrane, typically ranged from 10 nm to 100nm, can be controlled by the type of electrolyte used to fabricate the alumina membrane.

An electrochemistry bath that used the alumina membrane as filter to separate solutions with concentration gradient was built to investigate its diffusion and pumping properties. Experimental results indicated that the ion diffusion and pumping mechanisms of a real cell can be artificially imitated by the inorganic porous alumina membrane.

## 1. INTRODUCTION

Cell is the basic unit in a living body that can completely perform the sign of life. Cell membrane is one of the major components of a living cell. The controlled and selective flow of ions through biological channels in cell membranes enables efficient communication of biological information within cells inside a living body [1]. Passive transport and active transport are two main manners of the ion transport. The driving forces of the passive transport and the active transport are diffusion and ion-pumping, respectively. Diffusion property allows ions to travel from higher concentration area to lower concentration area. On the contrary, ion-pumping transports ions against the direction of electrochemical potential with energy coming from the hydrolysis of ATP [2]. Diffusion property has been well-known. However, the ion-pumping mechanism has still been under investigated. Recently, the Feynman's Ratchet and Pawl theory has been adopted to characterize the ion-pumping principles [3-7]. In which, conical nanopores in polyethylene terephthalate (PET) and polymide foils were produced by track-etching technique [8] to biomimic the transports of ions against electrochemical gradient.

In contrast to polymers, the inorganic porous alumina membrane (AAO) [9-11] that can be easily fabricated by dipping an aluminum foil into an electrolytic bath in which the aluminum metal was electrically anodized was adopted to imitate the cell membrane. The diameters of the pores on the membrane, typically ranged from 10 nm to 100nm, can be controlled by the electrolyte in the electrolytic bath. In addition, porous alumina membrane possesses other advantages such as conveniently to fabricate large membrane with relatively low cost and having good mechanical and chemical properties.

## 2. FABRICATION OF POROUS ALUMINA MEMBRANE

The procedure to fabricate the porous alumina membrane [12-13] is well known and does not require expensive instrumentation. Figure 1 diagrammatically illustrates the fabrication procedures of the porous alumina membrane. The procedures include (*i*) electropolishing aluminum foil (99.9995% Al, 96% sulfuric acid, 85% phosphoric acid, and DI water with 1:1:1 ratio) (*ii*) 1st anodization (0.3M oxalic acid) (*iii*) etching (50% phosphoric acid) (*iv*) 2nd anodization (0.3M oxalic acid) (*v*) removing aluminum substrate ($CuCl_2$+HCl) (*vi*) removing barrier (6% phosphoric acid). Figure 2 shows the SEM images of the porous alumina membrane used in this research.

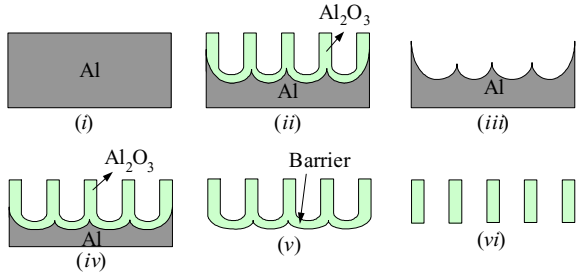


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Figure 1. Fabrication procedures of the porous alumina membrane

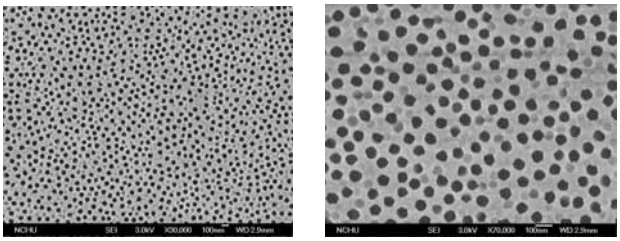

Figure2. SEM images of the porous alumina membrane used in this research

## 3. PREPARATION OF ION PUMP

An electrochemistry bath that used the porous alumina membrane as filter to separate solutions with different concentrations was built to conducting the experiments of ion diffusion and pumping. Figure 3 and 4 are the schematic diagram and real apparatus of the electrochemistry bath, respectively. Dimensions of the electrochemistry bath are 3.5 mm×2.5 mm×2.4 mm. The electrodes were made of copper.

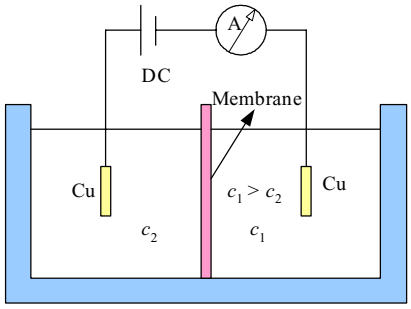


Figure 3. Schematic diagram of the electrochemistry bath

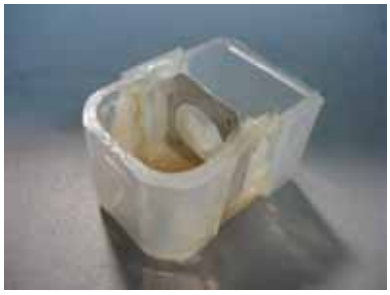

Figure 4. Real apparatus of the electrochemistry bath

## 4. EXPERIMENTS

During the experiments, diffusion and ion-pumping properties of a real cell membrane were imitated. KCl was the electrolyte solution. The CH1627A electrochemistry analyzer by CH Instruments was used to measure the ion currents.

(1) Diffusion experiments

Without externally electrical potential, the ion diffusion mechanism was investigated. The average diameter of the pores in the porous alumina membrane is about 60 nm. Figure 5 illustrates the current curves of different concentration gradients of KCl. It is found that both the current intensity and its variation gradient are proportional to the concentration gradient. Since the concentration gradient was the most at the beginning, the current intensity variations were more apparent during the first couple seconds. When the concentrations in each side of the electrochemistry bath were approaching equilibrium, the current intensity variations became inconspicuously.

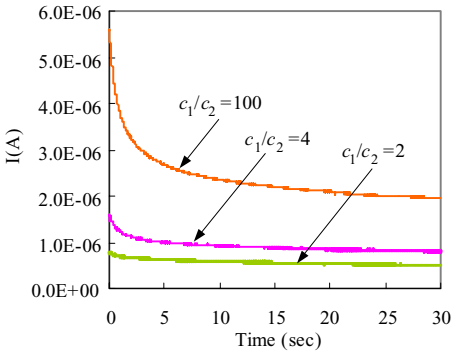


Figure 5. Current curves of different concentration gradients on diffusion experiments

In general, pores with diameter of 60 nm should allow both the cation ($K^+$) and anion ($Cl^-$) to penetrate simultaneously. i.e. the net current should be zero. Consequently, there should be other causes to the recorded current. One of the feasible reasons is the mobility difference between $K^+$ and $Cl^-$. $K^+$ navigates faster than $Cl^-$; therefore, net cations traveling through the membrane contribute to the recorded current. The electro-osmotic-flow effect inside the pores may be another possibility to the net current.

(2) Ion pumping experiments

When an electrical potential opposite to the concentration gradient was applied, the ion pumping mechanism was investigated. Figure 6 compares the diffusion and ion-pumping properties of the porous alumina membrane under 100-fold concentration gradient of KCl ($c_1/c_2$=100). The ion-pumping property was observed through the recorded against gradient current (Figure 6b).


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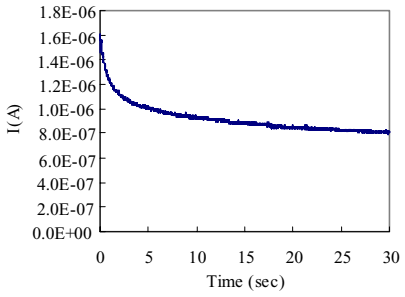
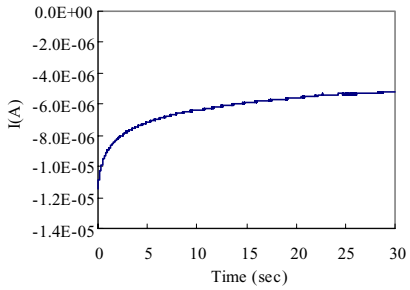



(a) Diffusion




(b) Ion-pumping (0.1 V)

Figure 6. Ion-pumping properties of the porous alumina membrane

Figure 7 schematically describes the ion-pumping mechanism. After a potential voltage being applied, cations in the lower concentration area penetrate the membrane toward the electrode of the higher concentration bath. On the contrary, anions in the higher concentration bath move to the electrode of the lower concentration area. Cations and anions are hereby separated. As time goes on, variation of the amount of the moving ions gradually decrease; therefore, the current intensity reaches its stable value (Figure 6b).

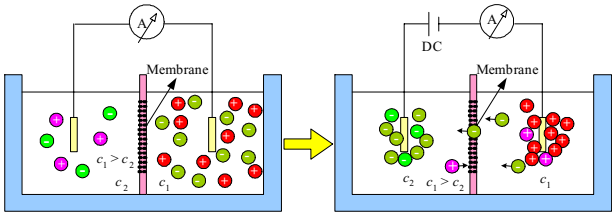
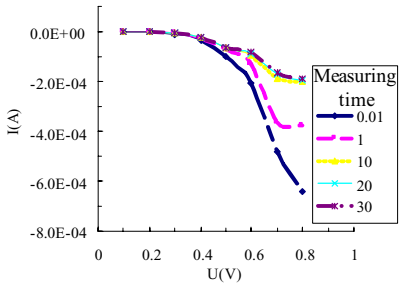


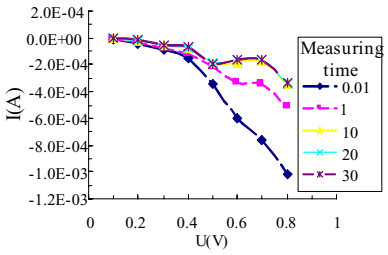

Figure 7. Schematic illustration of the ion-pumping mechanism

Figure 8 depicts the current intensities measured at different time intervals for different concentration gradient of the electrochemistry bath, under several oppositely electrical potentials.

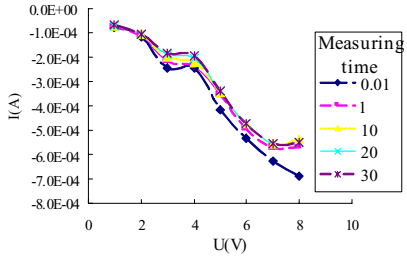
Experimental results show that the current intensity is proportional to the applied voltage. As observation time went on, the current intensities were getting smaller. It is also found that current intensity is inversely proportional to the concentration gradient. The reason is that the smaller the concentration of the solution is, the amount of ions inside the solution is rarer; therefore, the total amount of ions can contribute to the transport are fewer.




(a) $c_1/c_2$=100




(b) $c_1/c_2$=4




(c) $c_1/c_2$=1.333

Figure 8. Current intensities measurement for different concentration gradients

(3) Dependence of the pumping on the diameter of the pore

Porous alumina membranes with different pore diameters were fabricated to examine the dependence of the pumping property on the diameter of the pores. Figure 9 shows the relationship between the pumping property and the pore diameter. The recorded current is found to be proportional to the diameter of the pore.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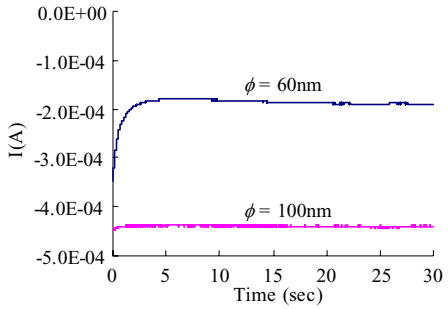


Figure 9. Dependence of the pumping property on the diameter of the pore

(4) Dependence of the pumping on the porous coverage

There kinds of membranes with different porous coverage were prepared to dig out the influence of the porous coverage percentage on the ion pumping property. The directly proportional dependence of the pumping property on the porous coverage percentage was illustrated in Figure 10.

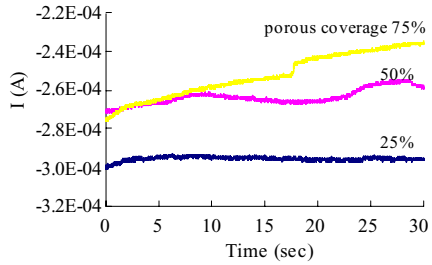


Figure 10. Dependence of the pumping property on the porous coverage

## 5. CONCLUSIONS

Diffusion and pumping of ions are two major mechanisms of the ion channels in real cell membrane. In this research, the porous alumina membrane was adopted to imitate the ion diffusion and pumping properties of a real cell membrane. The diameters of the pores on the membrane can be controlled by the electrolyte used to fabricate the membrane. Furthermore, the porous alumina membrane possesses other advantages such as conveniently to fabricate large membrane with relatively low cost and having good mechanical and chemical properties.

An electrochemistry bath that used the nanoporous membrane as the filter to separate solutions with concentration gradient was built to examine the diffusion and pumping properties of the alumina membrane. Without externally electrical potential, the ion diffusion mechanism was investigated. When an electrical potential opposite to the concentration gradient was applied, the ion pumping mechanism was investigated. Ion diffusion and pumping mechanisms of a real cell were artificially imitated through the experiments.

### 5.1. Subheadings

Subheadings should appear in lower case (initial word capitalized) in boldface. They should start at the left margin on a separate line.

*5.1.1. Sub-subheadings*

Sub-subheadings, as in this paragraph, are discouraged. However, if you must use them, they should appear in lower case (initial word capitalized) and start at the left margin on a separate line, with paragraph text beginning on the following line. They should be in italics.

## ACKNOWLEDGEMENTS

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